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A prospective cohort study of HPV-driven oropharyngeal cancers in the West of Scotland; implications for prognosis and immunisation

Running title: HPV-driven oropharyngeal cancers in the West of Scotland

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Abstract

Background: Human papillomavirus-driven oropharyngeal cancer is rising dramatically on a global scale. Contemporary data that provide insight into the prognosis of this disease in addition to the fraction attributable to HPV are essential to inform primary and secondary disease management strategies.

Methods: A population-based cohort of 235 patients diagnosed with OPC between 2013-15 in Scotland was assessed for HPV status using molecular genotyping. Associations between HPV status and key clinical and demographic variables were estimated using the Pearson chi-squared test. Rates of overall survival and progression-free survival were estimated and visualized using Kaplan–Meier curves.

Results: HPV DNA (largely HPV 16) was identified in 60% of cases. After adjustment for age, gender, deprivation, smoking, alcohol consumption and tumour stage, patients with HPV-positive OPC had an 89% reduced risk of death (HR = 0.11, 95% CI 0.05-0.25), and an 85% reduced risk of disease progression (HR = 0.15 95% CI 0.07-0.30). Those who reported excess alcohol consumption were less likely to be HPV positive.

Conclusions: The prevalence of HPV-associated OPC is high in Scotland and strongly associated with dramatically improved clinical outcomes including survival. The dominance of HPV 16 in OPC indicates the significant impact of prophylactic immunisation on this disease.

Background

Oropharyngeal cancer (OPC) is increasing globally and the rate has doubled in the past 15 years in the United Kingdom.[1-3] In Scotland, OPC is one of the most increasingly incident cancers – especially among men.[4] Tobacco use and alcohol consumption remain the major risk factors for head and neck cancers however, an increase in high-risk human papillomavirus (HR-HPV) driven OPC has also been observed.[5,6] Analyses of Scottish Cancer Registry head and neck cancer incidence trends over the past 40 years show that the rate of laryngeal cancer, which is strongly associated with tobacco consumption, has remained essentially stable. Oral cancer, which is associated with alcohol consumption, is steadily increasing, while OPC has increased comparatively more rapidly.[7] In relation to risk factors in head and neck cancer in Scotland these trends indicate a continued role of tobacco, an increasing role of alcohol, and a dramatic and relatively new role of HPV.[8]

The fraction of OPC associated with HPV varies significantly with geographic location; in the US prevalence is around 60% whereas in South America the attributable fraction is less than 5%.[9] A study of approximately 1500 archived OPC cases from 11 participating centres in the UK[10] (diagnosed between 2002 and 2011) showed approximately 50% were HR-HPV DNA-positive, largely for HPV type 16. Furthermore, the main HPV genotype identified in oral rinse samples from the general Scottish population was HPV 16.[11] HPV is thought to be acquired through sexual exposure [12,13] although the natural history of HPV-associated OPC is not as well defined as other HPV-associated neoplasms such as cervix. Although the increase in OPC incidence is of clear concern, patients with HPV-driven OPC generally have a better prognosis compared to those patients who are HPV-negative.[14] Of note, the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S) has developed a new staging classification system informed by HPV-status which recognises the improved clinical outcomes; this has now be endorsed/incorporated into the 8th edition TNM classification. [14]

US studies suggest that the sociodemographic status of head and neck cancer patients may also be changing, with an increasing proportion of patients with OPC diagnosis being younger and more socioeconomically affluent.[15] This phenomenon is not mirrored in Scotland, since recent national cancer registry data show that patients from the most deprived areas consistently had the highest rates of OPC. [8] While the HPV status of OPCs is not available in Scottish cancer registry data, the association between deprivation and cancer rates may reflect the overwhelming influence of tobacco and alcohol consumption in head and neck cancer oncogenesis in Scotland.

Little is known on whether improved outcome associated with HPV-driven OPC is reflected in populations where rates of tobacco use and alcohol are particularly high, such as the West of Scotland. Pre-2013, HPV testing of OPC in Scotland was performed opportunistically at the treating clinician's request, thereafter it was offered as a service to all Scottish health boards via a reference laboratory facility. This evaluation is one of the first to assess outcomes in a prospective, population based cohort of OPC patients, where HPV genotyping has been performed by a standardised methodology in a centralised location. We present data on the prevalence of HPV in OPC in Scotland and the association with key social variables and clinical indicators/outcomes.

Methods

Dimension and characteristics of patient cohort

All OPCs, including sub-sites of the tonsil, base of tongue, soft palate, and pharyngeal walls diagnosed in the West of Scotland Cancer Network between April 2013 and June 2015 underwent pathology review and prospective HPV genotyping as part of routine clinical care. The Health Boards included in the present analysis covered the following locations: West Dunbartonshire; East Dunbartonshire; East Renfrewshire; Glasgow City; Inverclyde, Renfrewshire, Forth Valley, Lanarkshire, Ayrshire and Arran which cover a population of approximately 2.5 million people. All individuals accessing health care in Scotland are assigned a unique 10-digit number, which allows linkage of clinical, social and laboratory data. Sociodemographic and clinical data were extracted from the West of Scotland Cancer Network Head and Neck Cancer Quality Performance Indicators, which are collected by all health boards in Scotland and used to drive quality improvement in cancer care across NHS Scotland and clinical records. The variables collected were: Date of diagnosis - taken as date of initial diagnostic biopsy sample collection, age at diagnosis, cancer stage; 7th edition TNM classification (TNM), 8th edition TNM classification for HPV positive OPCs/International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S)[14] which takes into account HPV status, treatment modality, date of relapse if occurred and if applicable date and cause of death, smoking status (never v. ever) at time of diagnosis and self-reported alcohol excess. Alcohol excess is defined by NHS Scotland as consuming alcohol at >=21 units or more per week for men and >=14 units per week for women] or documentation of "heavy", "excessive" or "dependency" on alcohol within clinical records). In addition, area-based socioeconomic status was obtained - via the Scottish Index of Multiple Deprivation (SIMD) where 1 and 5 are the most and least deprived respectively. Data were extracted by treating clinicians and all patient identifiable data were removed prior to statistical analysis. Study governance and ethical considerations were through NHS Greater Glasgow and Clyde Research and Development Office, the Clinical Effectiveness Team and a data sharing agreement with the West of Scotland Cancer Network, NHS. Data was censored at November 2016

Nucleic acid extraction & HPV genotyping

The relevant formalin fixed, paraffin-embedded (FFPE) block was selected and a 10 µm section obtained for nucleic acid extraction. Nucleic acid extraction was performed using reagents within the DNA mini kit (Qiagen, Hilde Germany) with an adaptation to the protocol to maximise recovery of HPV DNA.[16] Subsequently, HPV genotyping was performed using the Optiplex HPV Genotyping test (Diamex, Heidelberg, Germany). This assay detects 24 HPV types including all established HR types and, as a check for specimen adequacy, incorporates a cellular housekeeping control (betaglobin). This assay was used for the recent UK prevalence study of 1500 OPC referred to earlier.[10] All testing was performed at a centralised reference laboratory.

Analysis of HPV status with clinical outcomes

Associations between HR-HPV status and clinical and demographic variables were estimated using the Pearson chi-squared test. Both unadjusted and adjusted odds ratios (ORs) for HPV positivity and 95% confidence intervals (CIs) were calculated using logistic regression. Linear trend test was performed to investigate whether there was an increasing trend in the risk of HPV positive OPC with increasing age. Rates of overall survival (OS) and progression-free survival (PFS) were determined and univariate comparisons visualized using Kaplan–Meier curves. The univariate impact of each variable on survival was measured using Cox proportional-hazards regression. An adjusted model was created to obtain the adjusted hazard ratios for HR-HPV status with adjustment of the following covariates: gender, age at diagnosis (<50, 50-59, 60-69, 70-79 and 80+), SIMD, smoking and alcohol status and TNM classification 7 of cancer. Subset analysis on the HPV+ patients were carried out to obtain univariate and adjusted hazard ratio for ICON stage.

All statistical analysis was carried out using R version 3.2.3. All variables with P-values <0.05 were considered to be statistically significant.

Results

Description of cohort

A total of 235 patients were diagnosed with OPC between April 2013 and June 2015. Technically valid HPV results were obtained for 229 patients with 6 excluded due to non- amplification of the cellular housekeeping gene. Only 22 % of the 229 OPC patients were female and the median age was 60 (IQR 54-69). Most of the cohort were resident in a deprived area (37.6% in SIMD1 and 21% in SIMD2). The majority reported having ever smoked tobacco (65 %) with 31% reporting drinking alcohol to excess (Table 1).

HPV positivity and association with extrinsic risk factors

HPV DNA was identified in 60% of the cases (137/229). Single infections with 16, 18, 33, 35 were identified in 127, 2, 1, and 1 case, respectively. The occurrence of multiple infections of 16/6, 16/59, 18/82, 6/16/33 and 18/59/82 all occurred in a single case each. All bar one of the HPV positive cases included at least 1 established HR-HPV type.

Influence of gender and risk factors are summarised in Table 1. Men were twice as likely as women to have an HPV positive tumour (OR 2.44, 95% CI 1.18-5.05, P=0.02). Those who reported drinking alcohol to excess were 70% less likely to be HPV positive (OR 0.30, 95% CI 0.14-0.63, P=0.001). HPV positivity was not associated with social deprivation as measured by SIMD (P = 0.2) or age (linear trend P = 0.7), or smoking status (P = 0.1).

HPV status and survival

A total of 35 patients were excluded from the survival analysis: one was lost to follow-up and 34 received treatment intent of best support palliative care as they were not expected to recover. Post-exclusions, the mean follow-up time of the remaining cohort after OPC diagnosis was 2.1 years (range from 19 days to 3.4 years). In total, 23% patients (45/194) died and 28% patients (55/194)

had disease progression during the follow-up period. One-year OS rate for HPV negative patients was 75.7% while for HPV positive patients OS was 95.2%. One-year PFS rate for HPV negative and HPV positive patients was 67.1% and 92.7%, respectively (Figures 1 and 2). One year OS rates for the patients who smoked was 86.4% compared with 90.2% for the patients who reported never smoking. One-year OS rate for the patients who drank alcohol to excess was 80.4% compared with 91.5% for those who did not. In our study, we observed an increase in the point estimates for the hazard of death and disease progression as TNM classification 7 increased, but using ICON stage for the HPV positive population was not discriminatory (Tables 2 & 3, A1).

Univariate analysis also showed that HPV negative status and alcohol consumption were significantly associated with increased risk of death (both P<0.001, Table 2). After adjustment for age, gender, SIMD, smoking and drinking status and tumour stage, patients with HR-HPV positive OPC had 89% reduction in risk of death (HR = 0.11, 95% CI 0.05-0.24, P<0.001, Table 2), and had 84% reduction in risk of disease progression (HR = 0.15, 95% CI 0.07-0.30, P<0.001, Table 3) compared with those with HPV negative OPC). The risk of death increased with age in the fully adjusted model. Patients who reported drinking alcohol to excess had 2.3 times the risk of death (HR OS = 2.33, 95% CI 1.06-5.18, P=0.04) compared with those who did not drink alcohol to excess.

Discussion

We have conducted a detailed population-based evaluation to examine the impact of demographic, behavioural, and viral factors associated with OPC in a context where both disease prevalence and non-viral risk factors (alcohol consumption and smoking) are high. We have demonstrated that HR-HPV prevalence among OPCs in the West of Scotland was 60% and that HR-HPV positivity was higher in males compared to females, and lower amongst those who reported drinking alcohol to excess. We also confirmed that in our population HR-HPV status was strongly associated with improved overall and progression-free survival. It was of interest that individuals reporting tobacco exposure were as likely to have cancers associated with HR-HPV as those individuals who reported no history of tobacco use - a finding at variance with other previously reported UK data.[17]

The Scottish population has historically had a high rate of smoking, with 42% of adults reporting as current or ex-regular cigarette smokers (as of 2014), exceeding 60% in the most deprived areas [18] Rates of the cancers associated with tobacco consumption, namely lung and laryngeal cancer remain high in Scotland, with the impact of tobacco control in this region yet to be fully realised for cancer prevention. The other common risk factor for head and neck cancer is excessive alcohol consumption and in Scotland consumption habits are high with one in five adults consuming alcohol at a harmful level (21 units or more per week for men and 14 units per week for women).[18] A recent study demonstrated significant differences between key lifestyle factors in women living in Sweden and in Scotland. Scottish women demonstrated a higher frequency of alcohol consumption, smoking, obesity, low vegetable consumption and sedentary lifestyle.[19] Research suggests that for OPCs, incidence increases with increasing alcohol consumption and smoking for HPV negative but not among HPV positive people.[20,21] We did not observe an inverse relationship between HPV positivity and smoking status unlike other studies[9] and this may have been affected by the high smoking rates and the socio-economic status of our study population (Table 1).

Scottish Cancer Registry data show that the incidence of OPC rate has increased nearly 3-fold in recent years and is projected to continue to rise at this rate. [4,8] In a systematic review of HPV prevalence in OPCs, an approximate 20% increase in HPV-driven cancer from pre-1995 to 2014 worldwide was reported.[22] In Europe the mean prevalence of HPV in OPC was 28% (18.2%, 37.7%) pre-1995 rising linearly to 49.5% by 2005-2014.[22] Scottish Cancer Registry data have also demonstrated increased incidence rate among other HPV-associated cancers: anus, penis, vulva and vagina, which have no associated screening programme.[23] The reasons behind the observed increase in HPV-associated diseases are not fully understood, although sexual behaviour is likely to be influential.[24] However, with respect to OPC, evidence would indicate that in the UK specifically, HPV infection may not be solely responsible for the rise in incidence: Schache et al described that OPC rates doubled between 2002 and 2011 with an age-standardised rate of 2.1 to 4.1 and that this may not be attributed to HR-HPV solely given that the proportion that tested HPV positive over this period remained relatively static at around 50%.[3]

Studies suggest that incidence of HR-HPV detection in OPCs varies widely depending on geographic location and period of time. We report rates of 49% among women and 62.4% among men, with an overall positivity of 60%. This is broadly in line with the UK based results of Schache et al[3] and reflects a more recent period of assessment. Beyond the UK, studies of HR-HPV prevalence in OPCs in the most recent decade reports range from 6.1% in Spain [25], 38% in the Netherlands[26] and 62% in Denmark.[27] US estimates are consistently higher at around 60-70%.[9] A confounder to cross-study comparison is the different methods by which HPV positive status is defined, which vary across studies and include nucleic acid amplification tests, immunohistochemistry with p16INK4a, *in-situ* hybridisation and combinations thereof. This said, it is unlikely that assay choice is solely responsible for the level of discordance between settings.

Country-specific data on the attributable fraction of HPV associated disease are important as they will inform the decisions surrounding the utility and cost effectiveness of HPV vaccination including gender neutral vaccination. Certainly, significant impact of the vaccine on HR-HPV prevalence and disease outcomes including high grade cervical intraepithelial neoplasia (CIN2+) has already been observed in young women attending for screening in Scotland.[28,29] In addition, evidence of herd protection in the UK population in unvaccinated women and in heterosexual males has also been observed.[30] As reports suggest that HPV vaccination induces HPV antibodies in the oral cavity it is reasonable to assume that the vaccine will ultimately have an impact on OPC incidence.[31,32] This may be realised more rapidly in a gender neutral programme, but in countries with high uptake female-only programmes herd effects are likely to mitigate the burden of HPV associated OPC in both sexes.[33] This is important given that men are more likely than women to have OPC, potentially due to their greater exposure to multiple major risk factors namely HPV, smoking and alcohol excess. In line with this, previous studies have shown a significantly higher prevalence of oral HPV infection in men compared to women [9,34]The additive oncogenic effects of the major risk factors may contribute to the disparity between the sexes in the prevalence of OPC.

We also report an inverse association between HPV positivity and excess alcohol consumption. No other factor including smoking, social deprivation or cancer stage was associated with viral status. This is an interesting and important observation as it indicates that in Scotland patient-related

demographic and clinical factors cannot be reliably used to predict the HPV status of patients OPCs. This led to the allocation of centralised funding for provision of HPV genotyping of OPC patients in Scotland as part of routine clinical care.

In the adjusted analysis HPV status was strongly associated with improvement in overall and progression free survival during a median of 2-year follow up. This is in keeping with other studies of OPC and increasingly, other HPV-associated cancers.[35,36] D'Souza et al (2016) recently reported an reduction in the hazard of death among HPV positive OPC of 0.34 compared to HPV negative cases.[37] The present data are consistent with previous studies in that HPV positive cancers tend to occur in younger patient. It has been hypothesised that improved prognosis in HPV positive cancers may be due to better response rates to treatment through increased sensitivity to radiation and host immune response to the tumour.[38] While treatment for HPV positive and negative OPCs remains the same in many settings, the results of de-intensifying treatment trials in HPV positive OPC will be informative for future evidence-based management strategies[39]

This difference in survival and age and heavy alcohol consumption has implications for the ability to deliver and tolerate curative treatment, which may impact survival. Cause of death was out with the scope of this study but nutritional factors and co-morbidities in these patient groups should be further evaluated. In our data TNM classification 7 was more predictive of outcome than the new staging system that takes into account HPV status, although numbers were small. The British Association of Head and Neck Oncologists (BAHNO) have raised concerns of the use of TNM 8 and have suggested TNM 7 continues to be recorded. While the new staging system reflects improved understanding of cancer biology and clinical outcome of HPV positive OPCs further research is required to define if TNM 8 is generalisable to all populations and can be used to change treatment decisions.

Conclusion

In this evaluation of OPC patients in whom the prevalence of traditional risk factors of drinking and smoking was high; 60% of tumours were HPV positive. As a highly morbid disease, even if treatable, it is hoped that improved strategies for management and treatment, which are likely to be informed by HPV status, emerge. Furthermore, primary prevention through HPV immunisation is likely to reduce the incidence of oropharyngeal cancer in time although this could occur more quickly if gender neutral vaccination was in place, particularly if vaccine uptake in females declines. Our findings could further inform health economic modelling relevant to gender neutral immunisation. In the shorter term, Scotland must address other interventions to promote healthy living in order to reduce excessive alcohol consumption and smoking. Such interventions if successfully which would have far-reaching benefits beyond the reduction of OPC.

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